ELSEVIER

Journal of Cystic Fibrosis 10 (2011) 318–325

Journal of Cystic Fibrosis

www.elsevier.com/locate/jcf

Original Article

Chronic pulmonary infection with *Stenotrophomonas maltophilia* and lung function in patients with cystic fibrosis

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Received 8 December 2010; received in revised form 1 March 2011; accepted 9 March 2011
Available online 3 April 2011

Abstract

Background: The clinical consequences of chronic *Stenotrophomonas maltophilia* infection in cystic fibrosis (CF) patient are still unclear.
Method: All patients treated in the Copenhagen CF centre (N=278) from 1 January 2008 to 31 December 2009 were included. Each patient chronically infected with *S. maltophilia* for at least 2 years without any other chronic Gram-negative infection were matched to two non-infected CF controls.
Results: Twenty-one patients were chronically infected with *S. maltophilia* during the 2-year study period. Fifteen were infected for at least 2 years. The patients in the *S. maltophilia* group had a steeper decline (−3.2%/year vs. −0.3%/year) in FEV_1 compared to the non-infected CF controls (P=0.03). The rate of decline was the same as observed 3 years before the patients became chronically infected.
Discussions: Chronic infection with *S. maltophilia* does not lead to a steeper decline in lung function when compared to the period before chronic infection.

Keywords: *Stenotrophomonas maltophilia*; Cystic fibrosis; Lung infection

1. Introduction

Patients with cystic fibrosis (CF) are often colonised with opportunistic bacteria, causing bronco-pulmonary infections that lead to deterioration in lung function and in the end premature death [1,2]. The main bacterial pathogen is *Pseudomonas aeruginosa* [3] but other Gram-negative bacteria such as *Burkholderia cepacia* complex, *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* are emerging pathogens, and some with increasing prevalence [4–6]. The chronic infection with both *A. xylosoxidans* and *B. cepacia* complex has been shown to cause a significant decline in lung function [4,7].

Data regarding the multi-resistant Gram-negative rod *S. maltophilia* in CF patients are conflicting. Some authors have recorded a decline in lung function in infected patients [8–10] whereas others found no influence on the lung function [11–13]. Newer data suggest that patient-to-patient transmission may occur [14,15], and that patients chronically infected with *S. maltophilia* is cable of rising a specific antibody response against this bacteria associated with worse lung function [16].

S. maltophilia is isolated from CF patients of all ages and the U.S. Cystic Fibrosis Foundation's National Patient Registry reported a stationary infection rate in all age groups around 10–15% in 2008 [17]. A German study running over 6 months,

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doi:10.1016/j.jcf.2011.03.006

including 1419 CF patients found 31 patients who were cultured positive for *S. maltophilia* for the first time [18].

Since the clinical consequences of the infection still remain unclear, the purpose of the present study is to evaluate the consequences on lung function parameters in patients chronically infected with *S. maltophilia* as well as the prevalence and incidence of the bacterium in our clinic.

2. Materials and methods

2.1. General care

In this historical cohort study all CF patients (N=278) treated at the Copenhagen CF centre in the period from 1 January 2008 to 31 December 2009 were included. The patients are seen once a month in the outpatient clinic for evaluation of their clinical status. Sputum samples from the lower respiratory tract are collected for microbiological examination, pulmonary function tests are performed using a MasterLab Jäger, Würtzburg, Germany spirometer, and BMI, and height is measured. At least once a year, often more, precipitating antibodies against *P. aeruginosa*, *A. xylosoxidans*, *B. multivorens* complex and many other CF-related bacteria are measured [19].

Patients with chronic *P. aeruginosa, B. cepacia* complex and *A. xylosoxidans* are cohort isolated from other CF patients in the outpatient clinic, in the wards as well as at social events such as summer and winter camps to prevent patient-to-patient transmission [20,21].

All patients are treated according to fixed guidelines including PEP mask and daily administration of Pulmozyme (recombinant human DNase, Roche, Basel, Switzerland) [4].

Patients with chronic lung infections are treated with daily nebulised/inhaled and sometimes also oral antibiotic according to resistance of the bacteria and receive i.v. antibiotics for 2 weeks every 3 months as previously described [4,20]. Patients with chronic *S. maltophilia* infection are only treated if they show sign of exacerbations and appropriate antibiotics are used based on susceptibility testing.

2.2. Calculation of prevalence and incidence

Sputum and lower respiratory tract secretion were obtained by coughing or endo-laryngeal suction. The samples were Gram-stained and examined by microscopy and culture. The numbers of respiratory samples positive for *S. maltophilia* in the 2-year period were counted.

We have applied same definitions for *S. maltophilia* chronicity as for *P. aeruginosa* in our clinic, the patient are chronically infected if *S. maltophilia* have been cultured every month in 6 consecutive months, or less often when combined with an increase in number of specific, precipitating antibodies [22,23].

The yearly prevalence of colonised patients was calculated as the number of patients with one or more positive *S. maltophilia* samples divided by the number of patients treated at the centre this year. The yearly incidence is calculated as the number of patients who harboured *S. maltophilia* that year, and were not chronically infected the year before. The yearly prevalence of chronically infected patients was calculated as the number of patients who harboured the bacteria chronically that year divided by the whole population (N=278). To see if the incidence and prevalence was increasing, the yearly prevalence of colonised patients, the prevalence of chronically infected patients and the incidence were calculated from 2003 to 2009.

2.3. Impact of chronic S. maltophilia infection on the clinical course of the CF lung disease

Patients chronically infected with *S. maltophilia* for at least 2 years, who did not have any other chronic pulmonary Gram-negative infections, were matched to two CF controls with no chronic Gram-negative infection in the control period. The controls were matched by age (±3 years), gender and interval of forced expiratory volume in 1 s in percent of predicted (FEV_1%). The patients were divided into the FEV_1% of predicted intervals according to the mean FEV_1% of predicted the year they became chronically infected. The FEV_1% of predicted intervals were 25–40%, >40–60%, >60–80% and >80%. Because of age or infection status it was only possible to find matched controls to fourteen of the fifteen patients in the *S. maltophilia* group. Two controls were found to twelve of the patients in the *S. maltophilia* group whereas only one control was found to two of the patients in the *S. maltophilia* group. In total twenty-six matched controls were found.

To see if any decline in lung function could be assigned to the chronic *S. maltophilia* infection the change in lung function in the *S. maltophilia* group were also compared to the change in lung function in a period 3 years before they became chronically infected, the same was done in the CF control group as previously described in a study investigating the influence of *A. xylosoxidans* infection on the lung function [4]. Two patients in the *S. maltophilia* group did not have any FEV_1% data prior to infection as well as three pairs of controls because of young age and were therefore not included in that analysis, but the patient to whom it was not possible to find a control was included in this analysis. The change in lung function was determined as the slope (change in FEV_1% of predicted per year) of a linear regression in FEV_1% of predicted measured once a month. If two or more measurement a month were made, the mean of these values were used.

2.4. Measurement of specific anti-S. maltophilia antibodies

Precipitating antibodies were measured by crossed immunoelectrophoresis, as previously described [2,20,22,23]. We defined normal values as precipitating antibodies between 0 and 1 precipitins [22]. We investigated whether there is a correlation between a high level of precipitating *S. maltophilia* antibodies, and a decline in lung function, as previous described for *P. aeruginosa* [23] and *A. xylosoxidans* [2,4].

2.5. Co-infections

To see if any co-infections could have an influence on the change in lung function, the co-infections in the *S. maltophilia* group were compared to the co-infections in the CF control group before and during chronic infection. The co-infections were calculated as the percent of all cultures positive for a certain bacteria.

2.6. Typing of S. maltophilia

To investigate if any of the patients shared the same clone of *S. maltophilia,* indicating cross-infection, pulsed-field gel electrophoresis (PFGE) was done on the latest isolate of *S. maltophilia* from all 21 chronically infected patients. We used *Dra1* and *Xba1* for restriction enzymes as previously described [20,21] and compared PFGF patterns [21]. Due to lack of samples, we did not investigate if the patients had the same clone during the time of chronic infection or if re-infection occurred therefore, we cannot state whether the infection was caused by same clone, and thereby chronic, or if re-infection occurred with a new clone. Investigations on patients chronically infected with *P. aeruginosa* have shown that our definition of chronic infection is very reliable in sputum producing patients and chronically infected patients keep the same genotypes for longer periods of time [24]. Based on this we think that the infection with *S. maltophilia* is therefore most likely to be chronic [22].

2.7. Statistical analysis

Students t-test were used to see if there were any difference between age and FEV_1% of predicted in the *S. maltophilia* group and the CF control group. The statistical analysis on the change in FEV_1 per year was done using unpaired student's t-test when patients in the *S. maltophilia* group were compared to CF controls and paired student's t-test when patients in the *S. maltophilia* group or CF control group were compared to themselves. Level of significance was $P \leq 0.05$ (two-tailed).

To see if there was a difference in the number of patients with other infections than *S. maltophilia* in the two groups we used Fisher's exact test.

A linear regression analysis was used to see if any correlation between a high level of precipitating *S. maltophilia* antibodies, and a decline in lung function could be found. Additionally, it was tested whether the slope was significantly different from zero. All the statistical analysis and figures are made using GraphPad Prism version five to Windows 7.

3. Results

3.1. General care, incidence and prevalence

From 2008 to 2009 eighty-two CF patients (30%), had at least one positive culture for *S. maltophilia* with a median of tree positive cultures per patient (range 1–27). Further characteristic of patients with at least one positive *S. maltophilia* culture from 2008 to 2009 are shown in Table 1.

In Fig. 1 the yearly prevalence of *S. maltophilia* infected patients (a), the yearly prevalence of chronically *S. maltophilia* infected patients (b) and the yearly incidence of *S. maltophilia* infected patients (c) from 2003 to 2009 are shown. The yearly prevalence in the period was stable around 23 to 25%. The yearly prevalence in 2008 was 23%, 95% CI (0.19–0.29) and 21%, 95% CI (0.17–0.27) in 2009, respectively. The prevalence of chronically infected *S. maltophilia* patients varied from 4%, 95% CI (0.02–0.07) in 2003 to 7%, 95% CI (0.04–0.10) in 2009. The yearly incidence from 2003 to 2009 varied from 22%, 95% CI (0.18–0.28) in 2006 to 16%, 95% CI (0.12–0.21) in 2009 with a mean of 19%.

At the end of the study period (2008 to 2009) twenty-one patients had been chronically infected with *S. maltophilia* (median age 20 years (range 9–45 years)). Three of the twenty-one chronically infected *S. maltophilia* patients were also chronically infected with *P. aeruginosa;* these were excluded, as we wanted to see if chronic *S. maltophilia* infection by itself could lead to a decline in lung function. Three had been chronically infected for less than 2 years, and in order to evaluate the long-time importance of *S. maltophilia* these patients were not included in our analysis. Data were collected for the fifteen patients who had been chronically infected for 2 years or more, (median 42 months (range 24–107 months)) and did not have any other Gram-negative chronic infections during the time of *S. maltophilia* chronic infection, none of the fifteen patients got rid of the chronic infection during that period.

The age and lung function of the *S. maltophilia* infected CF patients and their CF controls are shown in Table 2.

No significant difference according to age, pancreatic insufficiency, nutritional status and lung function in the two groups at the beginning of the study period could be found.

Table 1
The number of cystic fibrosis patients with *S. maltophilia* positive cultures from 2008 to 2009.

	Number of patients with *S. maltophilia*/total	Number of *S. maltophilia* positive cultures/total [a]	Median number of *S. maltophilia* positive cultures per patient (range)	Median age (range)
Patients with ≥1	82/278	499/1877	3	19
S. maltophilia positive cultures	(30%)	(27%)	(1–27)	(2–60)
Chronic	21/278	341/501	16	20
S. maltophilia infection	(8%)	(68%)	(4–27)	(9–45)

[a] Number of *S. maltophilia* positive cultures divided by the total number of cultures in the eighty-two patients with one or more positive cultures, and in the patients chronically infected with *S. maltophilia*.

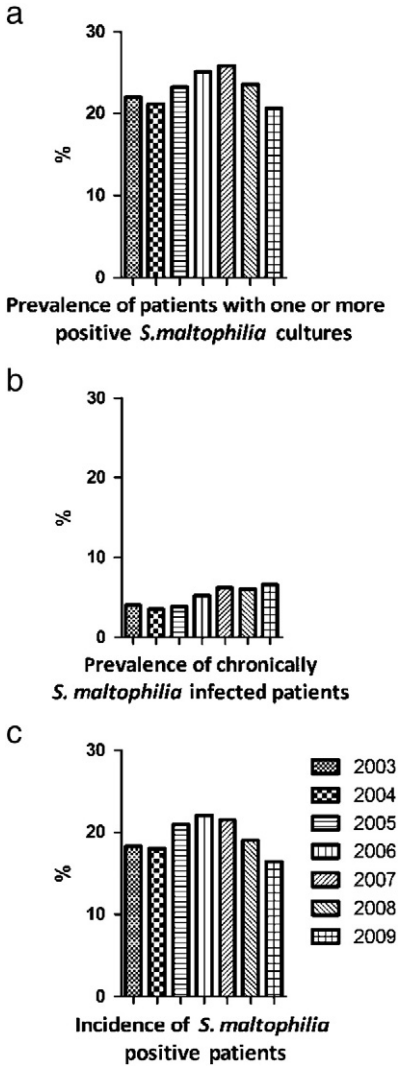

Fig. 1. The prevalence and incidence of *S. maltophilia*. The prevalence and incidence of *S. maltophilia* positive patients per year treated at the Copenhagen CF centre from 2003 to 2009. The prevalence of patients with one or more positive *S. maltophilia* cultures (a), the prevalence of chronically *S. maltophilia* infected patients (b) and the incidence of patients with one or more positive *S. maltophilia* cultures (c).

3.2. Impact of chronic S. maltophilia infection on the clinical course of the CF lung disease

When the *S. maltophilia* group were compared to the CF control group during the time of chronic infection a significant difference in FEV_1% of predicted per year were found (P = 0.03). The patients in the *S. maltophilia* group had a significantly larger decline in FEV_1 compared to the CF control group. The mean change in FEV_1 per year in the *S. maltophilia* group was −3.2% of predicted per year (range −7.0 to +2.2) (SD 2.7) and −0.3% in the control group (range −9.2 to +10.5) (SD 4.4).

When the patients chronically infected with *S. maltophilia* were compared to themselves in a period 3 years before they became chronically infected, no significant change in FEV_1 per year was found. The mean change in FEV_1% of predicted in the 3-year period before chronic infection was −2.2% of predicted per year (range −9.8 to +9.2) (SD 5.5) and −2.5% of predicted per year (range −6.4 to +2.2) (SD 2.4) during the time of chronic infection.

When the control group was compared to themselves 3 years before, no significant change in lung function was found (data not shown).

Table 2
Demographic data in CF patients with chronic *S. maltophilia* infection and in CF controls with no chronic Gram-negative infections.

	Chronically infected *S. maltophilia* patients	CF controls	P-value
Number (males)	14 (8)	26 (15)	
Median age (years)	14 (range 6–30)	11 (range 4–29)	n.s.
Median FEV_1% of predicted at start	84 (range 48–116)	92 (range 48–126)	n.s.
Mean BMI (range)	17.8 (14.0–23.8)	17.7 (14.8–23.9)	n.s.
Class 1 or 2 mutation	12	20	n.s.
Pancreas sufficient	0	2	n.s.
Diabetes mellitus	3	3	n.s.
MBL values. Low or insufficient (YA/0, XA/0 or 0/0)	4	5	n.s.

Three of the 21 chronically infected patients were also chronically infected with *P. aeruginosa* and were therefore excluded them from the analysis. Three other had been chronically infected for less than 2 years and were therefore excluded. Due to age and infection status it was only possible to find matched controls to fourteen of the included fifteen patients in the *S. maltophilia* group.

3.3. Co-infections

We investigated if any difference in co-infections could explain the decline in lung function; patients chronically infected with *S. maltophilia* had significantly more *Aspergillus* (calculated as the percent of samples positive for *Aspergillus* per patient) compared to the CF control group during time of chronic infection, (mean 32% range 2% to 100%) (SD 33.6) of all sputum samples in the *S. maltophilia* group and (mean 11% range 0 to 98%) (SD 24.1) of all sputum samples in the CF control group, (P = 0.03).

In the period 3 years before chronic infection, the tendency was the same although not formally significant (P = 0.06) (mean 29% range 0% to 100%) (SD 36.6) in the *S. maltophilia* group and (mean 11% range 0% to 88%) (SD 22.7) of all cultures in the CF control group.

Also, significantly more patients (14/14) (100%) had one or more cultures positive for *Aspergillus* compared to the CF control group (15/25; 60%, P = 0.007) during time of chronic *S. maltophilia* infection. Also here the tendency was the same 3 years before chronic infection (10/14) (71%) in the *S. maltophilia* group and (10/25) (40%) in the control group although not significant.

Significantly more patients in the *S. maltophilia* group (4/14) vs. (0/25) in the CF control group had one or more cultures positive for nontuberculous mycobacteria (NTM) (P = 0.01). Two patients were NTM culture positive before they became chronically infected with *S. maltophilia* and two during time of chronic infection.

Significantly more patients in the *S. maltophilia* group had been diagnosed with allergic broncopulmonary aspergillosis (ABPA, 7/14) compared to the CF control group (2/25; P = 0.004). These analyses were made using Fisher's exact test.

It was as previously mentioned only possible to find controls to 14 of the 15 cases, and one of the controls did not have any microbiology data 3 years before, and was therefore not included in this analysis.

No significant difference was found according to *P. aeruginosa* isolations between the two groups before chronic infection (P = 0.25), and during time of chronic infection (P = 0.5).

3.4. Typing of S. maltophilia

In order to investigate if *S. maltophilia* cross-infections occurred among the patients, PFGE to test the clonality was made on strains from the latest isolate from each of the 21 chronically infected patients. None of the patients shared the same strain, also showed before, indicating that no cross-infection has occurred, Fig. 2.

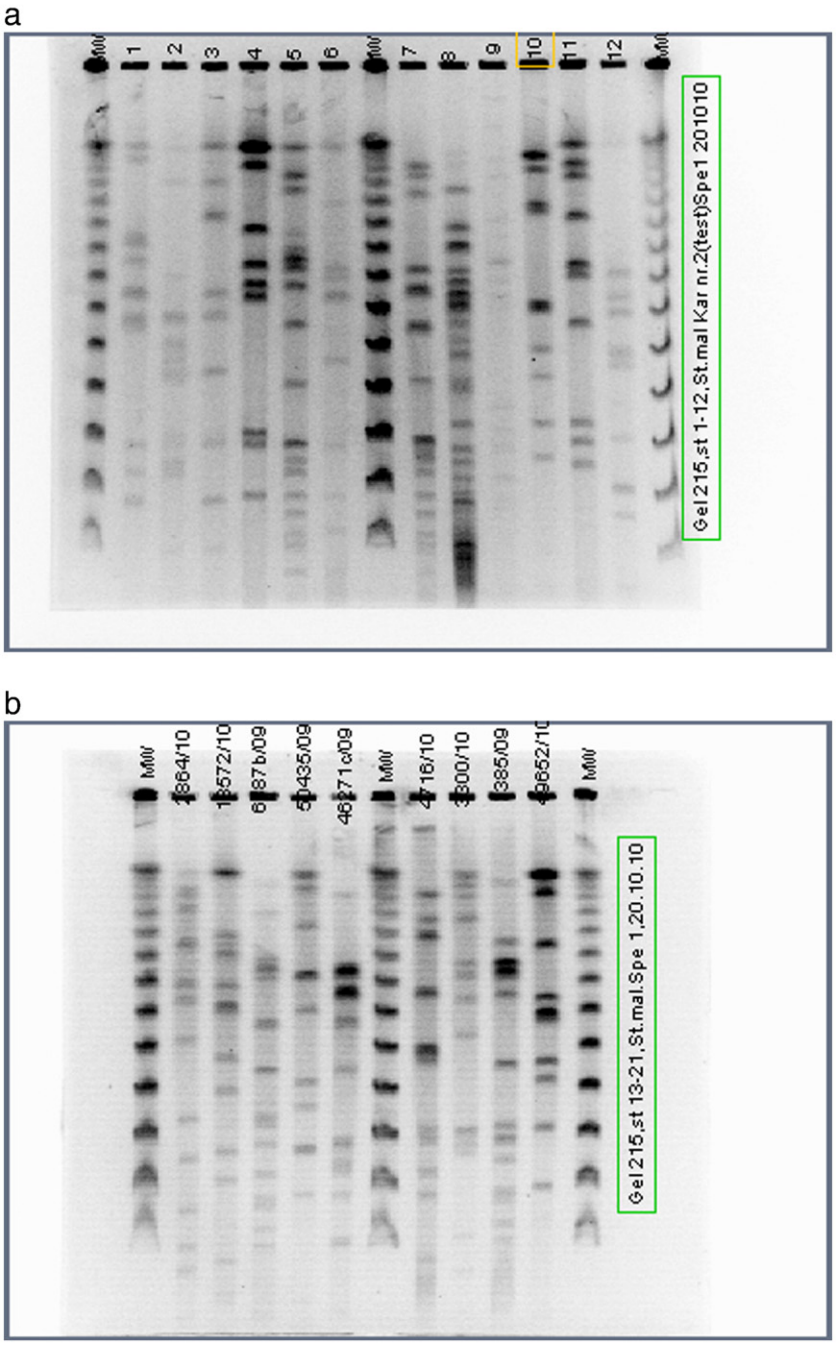

Fig. 2. Pulsed-field-gel-electrophoresis (PFGE) pattern of the *S. maltophilia* strains from the 21 chronically infected patients.The isolates from patient 1–12 (a) and the isolates from patient 13–21 (b). MW = molecular weight.

3.5. Specific anti-S. maltophilia antibodies

The level of precipitating antibodies against *S. maltophilia* increased from a median of 1 precipitin during the first year of chronic infection (range 0–11), to 6 in the second year of chronic infection (range 0–19) and 6 in the third year (range 0–18).

In the control group the level of precipitating antibodies remained normal (0–1).

3.6. Correlation between levels of specific anti-S. maltophilia antibodies and the change in lung function

A subgroup of patients chronically infected with *S. maltophilia* (N=4) had a rapid increase in the level of precipitating antibodies against *S. maltophilia* to a level of at least 12 antibodies, median 16 (range 12–18) during the first 3 years of chronic infection.

No significant difference in FEV_1% of predicted per year before or during chronic *S. maltophilia* infection was found when the patients in the subgroup were compared to themselves or to the group with a slower increase in antibodies (N=8; Table 3). Three patients were only infected for 2 years and did not have measurable precipitating antibodies in the third year and were therefore not included in the analysis.

For all the patients in the *S. maltophilia* group it was tested whether high levels of precipitating antibodies were correlated with a larger decline in lung function, Fig. 3. High levels of precipitating antibodies were reached, but no correlation was found.

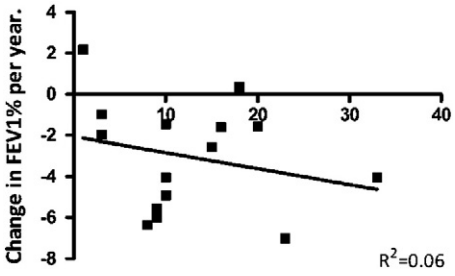

Fig. 3. Correlation between levels of precipitating antibodies and change in lung function. No correlation was found between a high level of precipitating *S. maltophilia* antibodies and a steep decline in lung function $R^2=0.06$ and P=0.37. ■, precipitating antibodies, normal values 0–1.

4. Discussion

We found a yearly prevalence of 24%, in 2008 and 21%, in 2009 of patients treated at the CF centre in Copenhagen with one or more *S. maltophilia* positive sputum cultures. In contrast to previously reported [5,6,25] no increasing prevalence was observed in our centre. But our yearly prevalence is higher than reported in other studies and centres elsewhere e.g. 4% by Millar et al. [6], 7% by Spicuzza et al. [25], 15% by Marchac et al. [11] and 12% by Emerson et al. [5]. The high prevalence in our centre could be because we obtain sputum samples from our patients on a monthly basis and thereby might detect a higher number of intermittently colonised patients.

Seven per cent of our patients are chronically infected with *S. maltophilia,* and it is therefore important to determine whether chronic infection with *S. maltophilia* has clinical consequences. A number of studies have investigated this topic, but the answers have been ambiguous. Some studies concluded that a positive *S. maltophilia* culture was associated with lower BMI and FEV_1 compared to *S. maltophilia* negative patients [13,26] and that patients with a *S. maltophilia* positive culture have more advanced disease [27]. Others could not find similar tendencies [12,28]. Demko et al. found that the 5-year survival of patients with severe pulmonary status was worse in *S. maltophilia* positive patients [29] and Karpati et al. that *S. maltophilia* chronically infected patients had a significantly decline in FEV_1 at 2 years follow up which was not found in patients chronically infected with *P. aeruginosa,* and they had significantly worse lung function compared to controls infected with *P. aeruginosa* [8].

None of these studies have compared patients with chronic *S. maltophilia* infection without other chronic Gram-negative infection to a CF control group without chronic Gram-negative infection.

We compared a group of chronically *S. maltophilia* infected CF patients to a CF control group with no chronic Gram-negative infections and found that patients who had been chronically infected with *S. maltophilia* for 2 years or more had a significantly larger decline in lung function expressed as change in FEV_1% of predicted per year when compared to the control group. However, when the patients in the *S. maltophilia* group were compared to themselves in the period 3 years before they became chronically infected this decline was also present at that time, indicating that the decline for some reason had already started before the patient became chronically infected.

A high level of precipitating antibodies or a steep increase has been associated with poor prognosis in patients chronically infected with *P. aeruginosa* [23] and *A. xylosoxidans* [4]. Waters et al. found that this also applied to *S. maltophilia* [16], but we did not find this association in patients with chronic *S. maltophilia* infection. The reason for this could be that patients have a higher age at onset of chronic *S. maltophilia* infection compared to *P. aeruginosa* [22], and that we looked at

Table 3
Change in FEV1% of predicted per year before and during chronic infection in the patients with a slow and fast increase in precipitating antibodies.

	Slow increase	Fast increase	P-value
Number of patients	8	4	
Included in the analysis	7 [a]	3 [a]	
Mean change in FEV_1% of predicted per year before chronic infection	−4.8 (range −9.8 to +3.7)	2.6 (range −2.2 to +9.2)	n.s.
Mean change in FEV_1% of predicted per year during chronic infection	−1.8 (range −4.9 to +2.2)	−2.7 (range −4 to −1.6)	n.s.
P-value	n.s.	n.s.	

[a] Two patients (one in each group) were not included while no data were available prior to infection because of young age.

the decline in lung function instead of FEV_1% of predicted at the time of serological examination.

We found that patients with chronic *S. maltophilia* infection had a higher level of *Aspergillus*, which was also found by Marchac et al. [11] and Valdezate et al. [30]. Furthermore, patients chronically infected with *S. maltophilia* seem to have more ABPA (50% of the patients vs. 8%), and more NTM compared to the controls. These co-infections may have contributed to the decline of lung function.

We found no evidence of cross-infection, indicating that isolation of the patients infected with *S. maltophilia* is not necessary, but of course a larger sample size must be investigated in order to exclude this possibility, and it would be interesting to see if a patient has the same clone during chronic infection or if infection with a new clone is occurring.

These results indicate that chronic infection with *S. maltophilia* is correlated to a decline in lung function, but this decline was already present prior to the chronic infection, where the high prevalence of *Aspergillus* and ABPA and NTM may have contributed.

This study has several limitations. The study population size is rather small, and a larger, multicentre study is needed to see whether our findings can be confirmed. Second, the controls were matched on an age range ±3 years, which is a rather large age span, and we were not able to find controls with the same rates of *Aspergillus* and NTM. Third, some of the patients had only been chronically infected for 2 years, which might not be long enough time to detect a possible adverse effect of the infection, It would therefore be very interesting to make a long-term follow up study.

In conclusion CF patients with chronic *S. maltophilia* infection experience a decline in lung function in contract to matched CF controls with no chronic Gram-negative infection. However, this decline of lung function was already ongoing before the onset of chronic *S. maltophilia* infection and may be due to a significantly increased prevalence of other infection e.g. *Aspergillus*, ABPA and NTM.

Although a prominent antibody response to *S. maltophilia* developed in some of the patients, this did not correlate to the decline of lung function observed during time of chronic infection. Long-term follow up studies are needed to evaluate the long-term outcome of chronic *S. maltophilia* infection.

References

[1] Pressler T, Frederiksen B, Skov M, Garred P, Koch C, Hoiby N. Early rise of anti-pseudomonas antibodies and a mucoid phenotype of *Pseudomonas aeruginosa* are risk factors for development of chronic lung infection—a case control study. J Cyst Fibros Jan 2006;5(1):9–15.

[2] Høiby N, Pressler T. Emerging pathogens in cystic fibrosis. In: Webb AK, Ratjen F, editors. Cystic fibrosis. European Respiratory Monograph. European Respiratory Society Journals Ltd; 2006. p. 66–78.

[3] Høiby N, Frederiksen B. Microbiology of cystic fibrosis. In: Hodson M, Geddes D, editors. 2 ed. London: Arnold; 2000. p. 83–107.

[4] Ronne Hansen C, Pressler T, Hoiby N, Gormsen M. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study. J Cyst Fibros Dec 2006;5(4):245–51.

[5] Emerson J, McNamara S, Buccat AM, Worrell K, Burns JL. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. Pediatr Pulmonol Apr 2010;45(4):363–70.

[6] Millar FA, Simmonds NJ, Hodson ME. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985–2005. J Cyst Fibros Dec 2009;8(6):386–91.

[7] Whiteford ML, Wilkinson JD, McColl JH, Conlon FM, Michie JR, Evans TJ, et al. Outcome of *Burkholderia* (*Pseudomonas*) *cepacia* colonisation in children with cystic fibrosis following a hospital outbreak. Thorax Nov 1995;50(11):1194–8.

[8] Karpati F, Malmborg AS, Alfredsson H, Hjelte L, Strandvik B. Bacterial colonisation with *Xanthomonas maltophilia*—a retrospective study in a cystic fibrosis patient population. Infection Jul-Aug 1994;22(4):258–63.

[9] Klinger JD, Thomassen MJ. Occurrence and antimicrobial susceptibility of gram-negative nonfermentative bacilli in cystic fibrosis patients. Diagn Microbiol Infect Dis Mar 1985;3(2):149–58.

[10] Ballestero S, Virseda I, Escobar H, Suarez L, Baquero F. *Stenotrophomonas maltophilia* in cystic fibrosis patients. Eur J Clin Microbiol Infect Dis Aug 1995;14(8):728–9.

[11] Marchac V, Equi A, Le Bihan-Benjamin C, Hodson M, Bush A. Case–control study of *Stenotrophomonas maltophilia* acquisition in cystic fibrosis patients. Eur Respir J Jan 2004;23(1):98–102.

[12] Gladman G, Connor PJ, Williams RF, David TJ. Controlled study of *Pseudomonas cepacia* and *Pseudomonas maltophilia* in cystic fibrosis. Arch Dis Child Feb 1992;67(2):192–5.

[13] Goss CH, Mayer-Hamblett N, Aitken ML, Rubenfeld GD, Ramsey BW. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. Thorax Nov 2004;59(11):955–9.

[14] Krzewinski JW, Nguyen CD, Foster JM, Burns JL. Use of random amplified polymorphic DNA PCR to examine epidemiology of *Stenotrophomonas maltophilia* and *Achromobacter* (*Alcaligenes*) *xylosoxidans* from patients with cystic fibrosis. J Clin Microbiol Oct 2001;39(10):3597–602.

[15] Marzuillo C, De Giusti M, Tufi D, Giordano A, Del Cimmuto A, Quattrucci S, et al. Molecular characterization of *Stenotrophomonas maltophilia* isolates from cystic fibrosis patients and the hospital environment. Infect Control Hosp Epidemiol Aug 2009;30(8):753–8.

[16] Waters V, Yau Y, Prasad S, Lu A, Atenafu E, Crandall I, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. Am J Respir Crit Care Med 2010 Oct 1.

[17] Registry CFFP, Report AD, Bethesda MCFF.

[18] Steinkamp G, Wiedemann B, Rietschel E, Krahl A, Gielen J, Barmeier H, et al. Prospective evaluation of emerging bacteria in cystic fibrosis. J Cyst Fibros Mar 2005;4(1):41–8.

[19] Johansen HK, Norregaard L, Gotzsche PC, Pressler T, Koch C, Hoiby N. Antibody response to *Pseudomonas aeruginosa* in cystic fibrosis patients: a marker of therapeutic success?—a 30-year cohort study of survival in Danish CF patients after onset of chronic *P. aeruginosa* lung infection. Pediatr Pulmonol May 2004;37(5):427–32.

[20] Johansen HK, Moskowitz SM, Ciofu O, Pressler T, Hoiby N. Spread of colistin resistant non-mucoid *Pseudomonas aeruginosa* among chronically infected Danish cystic fibrosis patients. J Cyst Fibros Sep 2008;7(5):391–7.

[21] Ojeniyi B, Frederiksen B, Hoiby N. *Pseudomonas aeruginosa* cross-infection among patients with cystic fibrosis during a winter camp. Pediatr Pulmonol Mar 2000;29(3):177–81.

[22] Hoiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey. Acta Pathol Microbiol Scand Suppl 1977(262):1–96.

[23] Hoiby N, Flensborg EW, Beck B, Friis B, Jacobsen SV, Jacobsen L. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. Scand J Respir Dis Apr 1977;58(2):65–79.

[24] Jelsbak L, Johansen HK, Frost AL, Thogersen R, Thomsen LE, Ciofu O, et al. Molecular epidemiology and dynamics of *Pseudomonas aeruginosa* populations in lungs of cystic fibrosis patients. Infect Immun May 2007;75(5):2214–24.

[25] Spicuzza L, Sciuto C, Vitaliti G, Di Dio G, Leonardi S, La Rosa M. Emerging pathogens in cystic fibrosis: ten years of follow-up in a cohort of patients. Eur J Clin Microbiol Infect Dis Feb 2009;28(2):191–5.
[26] Talmaciu I, Varlotta L, Mortensen J, Schidlow DV. Risk factors for emergence of *Stenotrophomonas maltophilia* in cystic fibrosis. Pediatr Pulmonol Jul 2000;30(1):10–5.
[27] Goss CH, Otto K, Aitken ML, Rubenfeld GD. Detecting *Stenotrophomonas maltophilia* does not reduce survival of patients with cystic fibrosis. Am J Respir Crit Care Med 2002 Aug 1;166(3):356–61.
[28] Graff GR, Burns JL. Factors affecting the incidence of *Stenotrophomonas maltophilia* isolation in cystic fibrosis. Chest Jun 2002;121(6):1754–60.
[29] Demko CA, Stern RC, Doershuk CF. *Stenotrophomonas maltophilia* in cystic fibrosis: incidence and prevalence. Pediatr Pulmonol May 1998;25(5): 304–8.
[30] Valdezate S, Vindel A, Maiz L, Baquero F, Escobar H, Canton R. Persistence and variability of *Stenotrophomonas maltophilia* in cystic fibrosis patients, Madrid, 1991–1998. Emerg Infect Dis Jan–Feb 2001;7(1): 113–22.